Synthesis of Certain Metabolites and Analogs of Vitamin B_6 and Their Ring-Chain Tautomerism

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Pyridoxol and pyridoxal on benzylation with dimethylphenylbenzylammonium hydroxide ("leucotrope") gave 3-*O*-benzylpyridoxol (IV) and 3-*O*-benzylpyridoxal (V), respectively. As a possible mechanism of this reaction an ion pair intermediate has been postulated. Oxidation of IV and V with chromic oxide-pyridine-acetic acid complex gave 3-*O*-benzyl-4-pyridoxic acid lactone (VI), which could also be obtained by benzylation of 4-pyridoxic acid. Treatment of VI with dimethylamine gave 2-methyl-3-benzyloxy-5-hydroxymethylpyridine-4-*N*,*N*-dimethylcarboxamide (X) which oxidized to form the 5-formyl derivative (XI). The latter on hydrolysis yielded the metabolite, 2-methyl-3-hydroxy-5-formylpyridine-4-carboxylic acid (I). When reacted with liquid ammonia, VI gave 3-*O*-benzyl-4-pyridoxamide (VII) which was then oxidized to give 2-methyl-3-benzyloxypyridine-4,5-dicarboxylic acid cyclic imide (IX). Acid hydrolysis of IX gave another metabolite, 2-methyl-3-hydroxypyridine-4,5-dicarboxylic acid (XIII), which could also be obtained by oxidizing XI with potassium permanganate in water to yield 2-methyl-3-benzyloxy-5-carboxypyridine-4-*N*,*N*-dimethylcarboxamide (XII) and subsequent hydrolysis with hydrochloric acid. A positional isomer of I, 2-methyl-3-hydroxy-4-formylpyridine-5-carboxylic acid (XVII) was synthesized starting from 3-*O*-benzyl-5-pyridoxic acid lactone (XIV) following similar reaction sequences used for the preparation of I. Ring-chain tautomerism has been studied in I, XVII, opianic acid (XVIII), phthalaldehydic acid (XIX) and (2-carboxy-4,5-dimethoxy)-phenylacetaldehyde (XX) in different solvents by nmr and in the solid state by ir spectroscopy. A direct and reliable differentiation between the open form (aldehyde proton in low field) and the ring form (lactol proton in the intermediate field) has been obtained by nmr spectroscopy. In sodium deuteroxide and pyridine-d_5 the open chain form existed exclusively (except for homolog (XX) which is in cyclic form in pyridine-d_5), whereas in 18% hydrogen chloride in deuterium oxide all the compounds are completely in the cyclic form. In hexafluoroacetone hydrate-d_2, XVIII, XIX, and XX exist in the cyclic form whereas I is in the open form. In DMSO-d_6 both cyclic and open-chain forms have been observed in XVIII, XIX and XX. Definite peak assignment for the two forms could not be made in I due to broadening or superimposition with C_6-H. The metabolite I, isometabolite (XVII) and opianic acid (XVIII) form cyclic acetyl derivatives which give a sharp lactol peak. In the solid state XVIII, XIX are in the cyclic form and I and XX in the open-chain form as observed by ir spectroscopy.

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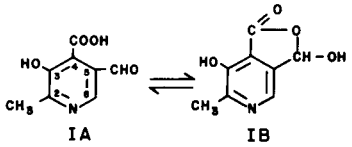
Catabolism of vitamin B_6 (1,2) which consists of pyridoxine, pyridoxal and pyridoxamine, proceeds by an oxidative attack on the 4 and 5 side chains of the molecules, depending on the type of organism studied. In higher organisms the 4-side chain is attacked first giving pyridoxal and 4-pyridoxic acid. For these metabolites we have developed direct methods of synthesis starting with pyridoxol (3).

In microbial systems the 5-side chain of the three forms of the vitamin is attacked preferentially, giving isopyrid-

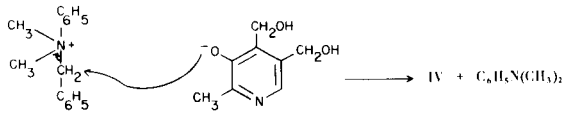
oxal and 5-pyridoxic acid, as well as products in which both side-chains are oxidized (4). While we have reported the synthesis of isopyridoxal and 5-pyridoxic acid earlier (5), a full account of the synthesis of the metabolite 2-methyl-3-hydroxy-5-formylpyridine-4-carboxylic acid I has not been published (6).



IA IB

This paper deals with the syntheses of this metabolite, and introduces general methods for other oxidative modifications of the 4- and the 5-positions, resulting in the syntheses of other metabolites and analogs of this vitamin. In addition we have studied the ring-chain tautomerism in I (A ⇌ B) and in related structures.

The starting point in these syntheses is the selective benzylation of the phenolic hydroxyl group of the different forms of the vitamin by means of dimethylphenylbenzylammonium hydroxide ("leucotrope") (7). Thus pyridoxol (II) and pyridoxal (III) could be benzylated by this reagent to yield the corresponding 3-*O*-benzyl derivatives IV and V, respectively (Scheme I), as the only products. In contrast to other alkylating agents (*e.g.* diazomethane) this reagent does not attack the pyridine nitrogen. The selectivity of the reagent towards the acidic hydroxyl groups (such as phenolic or carboxylic) can be explained by the formation of the ion pair immediately on the addition of the reagent as shown below.

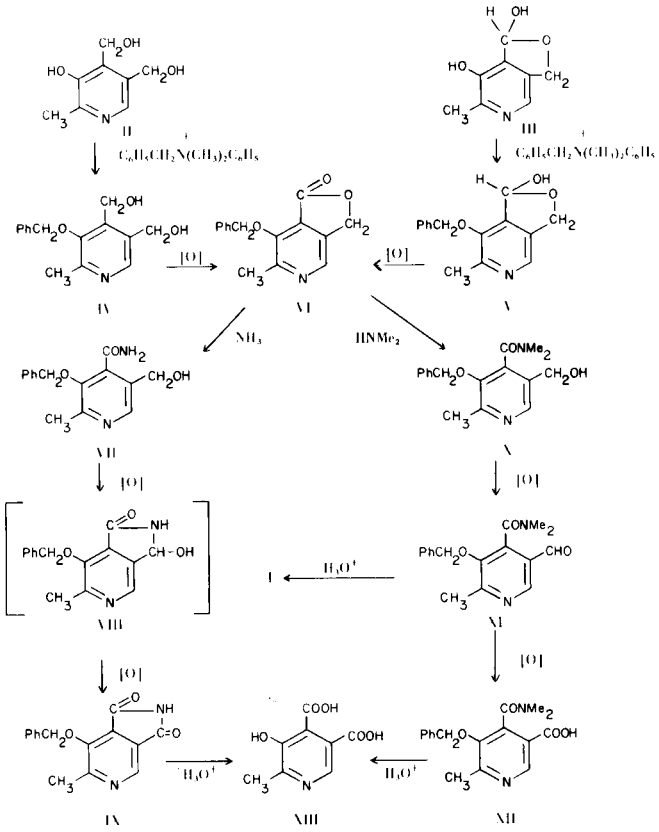


On heating the phenolate anion attacks the electron deficient benzylic CH_2 group to form IV.

Oxidation of compound IV or V with chromic oxide-pyridine-acetic acid complex (8) gave 3-*O*-benzyl-4-pyridoxic acid lactone VI. Hydrolysis of the lactone VI with acid gave the well-known metabolite, 4-pyridoxic acid lactone, in quantitative yield. This method compares favorably with that developed by Heyl (9) since the yields from the oxidation of IV and V were satisfactory (60 and 90%, respectively).

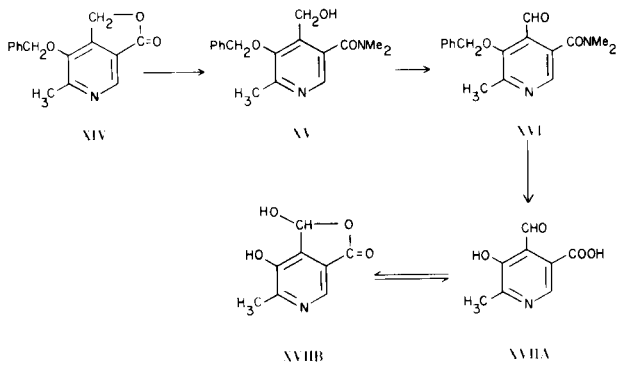
The lactone VI could be converted to the amide VII on treatment with liquid ammonia. Oxidation of the amide VII with chromium trioxide-pyridine-acetic acid gave the dicarboxylic acid imide (IX). The oxidation probably proceeds in two steps: initially, 2-methyl-3-benzyloxy-5-formylpyridine-4-carboxamide is formed, which presumably exists in the cyclic lactol form (VIII). The oxidation proceeds further, the secondary alcohol group in XIII being oxidized to give IX. Since the oxidation reaction could not be stopped at the lactol stage, we had to preclude its formation by the conversion of the lactone VI with dimethylamine into the corresponding dimethylamide X. Oxidation of this amide gave 2-methyl-3-benzyloxy-5-formylpyridine-4-*N*,*N*-dimethylcarboxamide XI, which on hydrolysis with 1*N* hydrochloric acid yielded the desired metabolite I. The synthetic material was identical in all respects with the naturally occurring compound kindly supplied by Dr. E. E. Snell (10).

Scheme I



A positional isomer of I, in which the carboxyl and aldehyde functions have been interchanged (XVII, designated as "isometabolite"), was prepared starting from 5-pyridoxic acid lactone, which was *O*-benzylated to yield XIV and converted to the dimethylamide XV with anhydrous dimethylamine. Oxidation of the latter with either chromic oxide-pyridine-acetic acid mixture or manganese dioxide gave the 4-aldehyde derivative XVI. Unlike in the case of the isomeric precursor XI of the metabolite, hydrolysis of XVI yielded an unstable product which decomposed on attempted purification. The product could be characterized as the cyclic diacetyl derivative of XVIIB and as a semicarbazone derivative of the open chain form XVIIA. The greater instability of the isomer

Scheme II



XVII is probably related to the generally found enhanced reactivity of the substituents in the 4 position in the pyridoxine molecule (such as 4-aldehyde group) as compared to those in the 5 position (12). An earlier attempt to synthesize XVII, from 3-*O*-benzylpyridoxaloxime by oxidation of 5-CH_2OH to 5-COOH gave a mixture of products.

2-Methyl-3-hydroxypyridine-4,5-dicarboxylic acid (XIII), a product of further metabolic degradation, was obtained by two methods (Scheme I). Acid hydrolysis (heating with 1*N* hydrochloric acid for 2 hours) of the acid imide (IX) gave the dicarboxylic acid (XIII) directly. The acid could be also obtained in two steps by oxidation (potassium permanganate in water) of the aldehyde (XI) to the acid (XII) and subsequent hydrolysis with hydrochloric acid. An alternative method for synthesis of the metabolite (XIII) has been reported previously (11). The present syntheses of the three metabolites (*i.e.* 4-pyridoxic acid lactone, I and XIII) demonstrate the utility of benzyl as a protecting group in a systematic approach to modification of the pyridoxol molecule, and make it possible to duplicate chemically, step by step, the enzymatic degradation of the vitamin.

Since further oxidation of the metabolite I of the dicarboxylic acid (XIII) is probably enzymatic, there is a question as to which of the two forms is the substrate for the enzyme. In this connection it should be pointed out that the lactol form (IB) has an asymmetric carbon atom, and that the oxidation of IB thus may well be stereoselective with regard to one of the two antipodes.

The general type of the ring-chain tautomerism IA ⇌ IB has been extensively studied with *o*-phthalaldehydic, especially 5,6-dimethoxyphthaldehydic (opianic), acid by chemical and spectroscopic methods, and the subject has been reviewed (13,14). We have examined the effects of solvents, *p*H and temperature on the ring-chain tautomeric equilibrium of the metabolite I and isometabolite XVII and compared with those of the much studied opianic acid (XVIII), phthaldehydic acid (XIX) and a homolog of opianic acid (2-carboxy-4,5-dimethoxy)phenylacetaldehyde (XX) (16) by nmr spectroscopy (Table I). This study was prompted by an occasional apparently inconsistent behavior of these compounds in different solvents. The differentiation between the open form (1A, aldehyde proton in low field) and the ring form (1B, lactol proton in intermediate field) can be readily made by nmr spectroscopy and the relative amounts of the two forms can usually be readily estimated in a tautomeric mixture by the integration of the two peaks. Thus, the metabolite I, isometabolite XVII, opianic acid XVIII, and phthaldehydic acid XIX in 1*N* sodium deuteroxide are in the open form exclusively as is evidenced from the presence of a one-proton aldehyde singlet at 9.77, 10.05, 9.80 and 9.92 ppm respectively. In pyridine-d_5, the metabolite I, opianic acid XVIII and phthalaldehydic acid XIX exists in open-chain form, whereas the opianic acid homolog XX is in the cyclic form (Table I). In strongly acid solution (6*N* hydrogen chloride in deuterium oxide) the aldehyde singlet is replaced by a lactol peak (δ 7.00-7.66 ppm) indicating that the compounds exist completely in the cyclic form. Thus conditions tending to favor the formation of the dissociated carboxyl group, such as high *p*H or zwitterion formation, stabilize formation of the open-chain form (IA), and at low *p*H the cyclic form (IB) is favored. It is interesting to note that this degree of solvent and *p*H dependence in ring-chain tautomerism is not present in an *o*-aldehyde hydroxymethyl system (*e.g.*, pyridoxal) which exists predominantly in the cyclic form in various solvents, even in very alkaline solutions (15). In hexafluoroacetone hydrate-d_2 the equilibrium was found by nmr to be completely in favor of the cyclic (lactol) form for opianic acid XVIII, phthalaldehydic acid XIX and (2-carboxy-4,5-dimethoxy)-phenylacetaldehyde XX and the open (aldehyde) form for the metabolite I. We explain this discrepancy by the

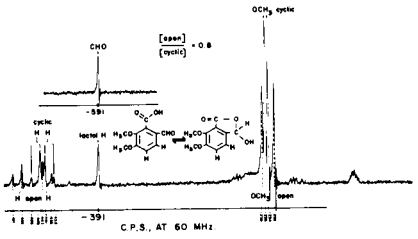


Figure 1. 60 MHz nmr spectrum of opianic acid (XVIII) in DMSO-d_6 at probe temperature. The dotted lines are for the open form and solid lines are for cyclic forms. Peak positions are shown in cps.

Table I

Nmr Spectra of 2-Methyl-3-hydroxy-5-formylpyridine-4-carboxylic Acid (I) and Related Compounds.

Compound	Solvent	Aldehyde *H*	Lactol *H*	Other *H*
Metabolite I	6*N* HCL in D_2O	---	7.37 (s)	8.65 (C_6-*H*), 2.85 (2-CH_3)
Isometabolite XVII	"	---	7.00 (s)	8.77 (C_6-*H*), 2.85 (2-CH_3)
Opianic Acid XVIII	"	---(a)	7.66	3.91 (5, 6-OCH_3), 7.40 (q, J = 8 cps, 3, 4-*H*s) (b)
Phthalaldehydic Acid XIX	"	---	6.77	7.68 (aromatic *H*s)
(2-Carboxy-4,5-dimethoxy)-phenylacetaldehyde XX	"	---	5.85	3.75, 3.82 (4, 5-OCH_3) 6.78, 7.18 (3, 6-*H*), 3.07 (CH_2)
I	1*N* NaOD	9.77 (s)	---	7.93 (C_6-*H*), 2.42 (2-CH_3)
XVII	"	10.05	---	7.33 (C_6-*H*), 2.35 (2-CH_3)
XVIII	"	9.80 (s)	---	3.92, 4.00 (5, 6-OCH_3) 7.45 (q, J = 8 cps, 3, 4-Hs) (b)
XIX	"	9.92 (s)	---	7.67 (m, aromatic *H*s)
I	Pyridine-d_5	11.37 (s)	---	8.62 (C_6-*H*), 2.72 (2-CH_3)
XVIII	"	11.73 (s)	---	3.75, 4.02 (5, 6-OCH_3), 7.38 (q, J = 8 cps, 3, 4-Hs) (b)
XIX	"	10.90 (s)	---	7.79 (m, aromatic *H*s)
XX	"	---	6.38 (m)	3.74, 3.78 (4, 5-OCH_3), 6.84, 7.82 (3, 6-H), 3.36 (m, CH_2)
I	$(CF_3)_2$ C=O, D_2O	10.67 (s)	---	8.17 (C_6-*H*), 2.76 (2-CH_3)
XVIII	"	---	7.42 (s)	3.97, 4.05 (5, 6-OCH_3) 6.95 (b, 3, 4-Hs)
XIX	"	---	6.52 (s)	7.78 (m, aromatic Hs)
XX	"	---	5.78	3.82, 3.84 (4, 5-OCH_3) 6.73, 7.46 (3, 6-H), 3.09 (CH_2)
I	DMSO-d_6	d	d	8.23 (C_6-*H*), 2.55 (2-CH_3)
XVIII	"	9.85 (s)	---	Open form: 3.80, 3.97 (5, 6-OCH_3), 7.53 (q, J = 8 cps, 3, 4-Hs) (b)
			6.52 (s)	Cyclic form: 3.90, 3.93 (5, 6-OCH_3), 7.38 (q, J = 8 cps, 3, 4-Hs) (b)
XIX	"	10.49 (s)	6.66 (s)	7.77 (m, aromatic Hs)
XX	"	7.71 (m)	5.82 (m)	3.80, 3.85 (4, 5-OCH_3) 6.96, 7.36, (3, 6-*H*) 3.08 (m, CH_2)
Acetyl derivative of I (c)	$CDCl_3$	---	7.45 (s)	8.72 (C_6-*H*), 2.58 (2-CH_3) 2.20, 2.45 (acetyl H)
Acetyl derivative of XVII (c)	"	---	6.85 (s)	8.87 (C_6-*H*), 2.57 (2-CH_3) 2.15, 2.35 (acetyl *H*)
Acetyl derivatives of XVIII (c)	"	---	7.30 (s)	3.95, 4.13 (5, 6-OCH_3), 7.25 (q, J = 8 cps, 3, 4-H) (b) 2.18 (acetyl H)

(a) Sample heated to 100° to increase solubility. (b) Center of an AB quadruplet. (c) Acetyl derivatives were prepared as described by C. Liebermann, *Ber.*, 19, 2275 (1886) for opianic acid. (d) The aldehyde and/or the lactol proton peak could not be definitely assigned.

existence of a zwitterionic structure in the metabolite I in which the carboxylic acid group is dissociated by the pyridine N (as an example p*K*a of isonicotinic acid 1.7 (COOH), N^+ H (4.9)) and hence cannot form a lactol ring. In the solid state the metabolite exists in the open chain form, as is indicated by a carboxyl absorption at 1719 cm^{-1} and aldehyde absorption at 1575(sh) cm^{-1} in its infrared spectrum in Nujol mull. Surprisingly the homolog of opianic acid XX was also found in the open-chain form as shown by its infrared spectrum (carbonyl absorption at 1673, 1600 cm^{-1}). In contrast, opianic acid XVIII, and phthaldehydic acid XIX are in the cyclic form, as is indicated by a single carbonyl absorption at 1760 cm^{-1}.

Other apparent discrepancies are related to the rate of interconversion of the ring-chain forms. Thus the nmr spectrum of opianic acid XVIII, phthalaldehydic acid XIX and the opianic acid homolog XX in DMSO-d_6 shows the characteristics of a mixture of the two forms; both the aldehyde and the lactol protons peaks are present, and the ratio of their areas is 0.8, 0.2 and 1.0, respectively. The nmr spectrum of opianic acid (XVIII) in DMSO-d_6 is shown in Figure 1 where both open and cyclic forms appear as separate entities. However, the nmr spectrum of the metabolite in the same solvent does not show the two ring-chain forms as individual entities, and the potential aldehyde proton appears as a broad peak at a position intermediate between the aldehyde and lactol positions. On heating DMSO-d_6 solutions of opianic (XVIII), phthal-aldehydic (XIX), and opianic homolog (XX) acids to 70°, the aldehyde proton peak disappears. On cooling to probe temperature the aldehyde peak reappears except in the case of the opianic acid homolog XX, which remains in the cyclic form after heating and then cooling to room temperature. Chemically both opianic acid XVIII and the metabolite I and isometabolite XVII behave analogously in forming cyclic and acyclic derivatives. Thus opianic acid XVIII as well as the metabolite I and isometabolite XVII forms cyclic acetyl derivatives, which give a normal sharp lactol peak in deuteriochloroform solution, whereas the oxime and semicarbazone of each compound are derivatives of the straight chain form.

EXPERIMENTAL

Melting points (uncorrected) were determined by the capillary method. Infrared spectra were determined with a Perkin-Elmer 137B or 457 spectrophotometer and nmr spectra were obtained with a Varian A60A or HA100 or XL100 instrument; positions of the peaks are expressed in δ (ppm) from TMS; for deuterium oxide solutions, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) or dioxane was used. Thin layer chromatograms were run routinely on Merck HF-254 silica gel plates and spots on chromatograms were detected by their uv absorption and Gibb's color test.

Isopyridoxal Diethylmercaptan.

Ethanethiol (5.25 ml.) was added slowly with stirring to an ice cold solution of isopropylidene isopyridoxal (280 mg.) in concentrated hydrochloric acid (0.8 ml.) and shaken at room temperature overnight, and evaporated completely *in vacuo*. The yellow residue was dissolved in 4-5 ml. of water, cooled in ice and neutralized with sodium bicarbonate, when a yellowish gum separated out, which on scratching and cooling solidified. It was crystallized from boiling hot water (*ca.* 10 ml.) after treatment with charcoal, m.p. 101-102°, yield 0.295 g. (77%); ir (Nujol): 2688 (OH), 724 (C-S), cm^{-1}.

Anal. Calcd. for $C_{12}H_{19}NO_2S_2$: C, 52.74; H, 6.95; N, 5.12; S, 23.44. Found: C, 52.66; H, 6.85; N, 4.86; S, 23.31.

3-*O*-Benzylpyridoxol (IV).

Pyridoxol hydrochloride (2.0 g.) was added to an alcoholic solution of sodium ethoxide prepared from sodium metal (0.5 g.) in 25 ml. of absolute alcohol, stirred at room temperature for one hour, and cooled in ice. A solution of dimethylphenylbenzyl-ammonium chloride (3.0 g.) in 25 ml. of absolute alcohol was added slowly to it with stirring. The mixture was stirred at room temperature for 2 hours and then evaporated completely *in vacuo*. Dry benzene was added to the residue twice and evaporated to remove traces of alcohol. Dry xylene (25 ml.) was added to the residue and heated gently to reflux for 3 hours, evaporated completely *in vacuo*, and water (25 ml.) was added to the residue and extracted several times with benzene. The combined benzene extracts were dried over calcium sulfate and evaporated *in vacuo*, and crystalline material separated out. After cooling the crystals were filtered, the residue washed with cold ether and dried, yield 1.43 g. (57%), m.p. 113-114° (after recrystallization from benzene), (hydrochloride m.p. 198-199°); ir (Nujol): 3401 (OH) cm^{-1}; nmr (deuteriochloroform): δ 2.45 (s, 2-CH_3), 4.65, 4.73 (2xs, 5CH_2, 4CH_2), 4.88 (s, 3-*O*-CH_2), 7.37 (s, C_6H_5), 8.02 (s, C_6H).

Anal. Calcd. for $C_{15}H_{17}NO_3$: C, 69.49; H, 6.56; N, 5.40. Found: C, 69.56; H, 6.65; N, 5.33.

3-*O*-Benzylpyridoxal (V).

This compound was prepared by the above procedure using pyridoxal hydrochloride (2.0 g.), sodium metal (0.5 g.), dimethylphenylbenzylammonium chloride (3.0 g.) and alcohol (60 ml.), yield, 1.56 g. (62%). The compound was crystallized from ether, m.p. 165-166°; ir (Nujol): 3509, 3344 (OH) cm^{-1}; nmr (deuteriochloroform): δ 2.48 (s, 2-CH_3), 5.23 (5CH_2), 5.30 (s, 3-*O*-CH_2), 6.65 (4CH), 7.38 (s, C_6H_5), 7.98 (C_6-H).

Anal. Calcd. for $C_{15}H_{15}NO_3$: C, 70.03; H, 5.83; N, 5.44. Found: C, 70.29; H, 5.79; N, 5.53.

3-*O*-Benzylpyridoxal Oxime.

3-*O*-Benzylpyridoxal (90 mg.) was dissolved in warm alcohol (5 ml.), and was cooled in ice. Sodium hydroxide solution (1*N*, 0.2 ml.) was added, followed by hydroxylamine hydrochloride (100 mg.). The solution was heated to reflux for one hour, cooled, and evaporated to dryness *in vacuo*. After the addition of water a solid material separated out, which was then filtered and dried; yield, 85 mg. (89%), m.p. 164-165° (after crystallization from ethyl acetate); ir (Nujol): 3257 (OH), 1613 (C=N) cm^{-1}.

Anal. Calcd. for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29. Found: C, 66.18; H, 5.91; N, 10.50.

3-*O*-Benzyl-4-pyridoxic Acid Lactone (VI).

A. From 4-Pyridoxic Acid.

A solution of sodium metal (0.28 g.) in absolute alcohol (10

ml.) was added slowly with stirring to an ice cold solution of dimethylphenylbenzylammonium chloride (3.0 g.) in absolute alcohol (20 ml.) and stirred for 10 minutes, and then added to an ice cold stirred suspension of 4-pyridoxic acid (1.0 g.) in absolute alcohol (20 ml.). After stirring at room temperature for 1 hour, the reaction mixture was evaporated to dryness *in vacuo*, dry benzene was added to the residue and the mixture was evaporated. This process was repeated to remove traces of alcohol. Dry xylene (25 ml.) was added to the residue and it was heated gently to reflux for 3 hours, the solvent evaporated, water (25 ml.) was added and the reaction mixture was extracted several times with ether. The combined ether extract was dried over calcium sulfate, evaporated to a small volume when crystalline material separated which was filtered and dried, yield, 0.91 g. (65%), m.p. 135-136° (after crystallization from ether); ir (Nujol): 1779 (C=O) cm^{-1}; nmr (deuteriochloroform): δ 2.52 (s, 2-C*H*$_3$), 5.33 (s, 5C*H*$_2$), 5.48 (s, 3-*O*-C*H*$_2$), 7.40 (s, C$_6$*H*$_5$), 8.40 (s, C$_6$-*H*).

Anal. Calcd. for $C_{15}H_{13}NO_3$: C, 70.58; H, 5.13; N, 5.49. Found: C, 70.66; H, 5.18; N, 5.47.

B. From 3-*O*-Benzylpyridoxal (V).

A mixture of 3-*O*-benzylpyridoxal (100 mg.) and chromic oxide-pyridine-acetic acid complex solution (8) (8 ml.) was shaken for 24 hours. After cooling in ice, the mixture was diluted with 25 ml. of ice cold water and extracted several times with ether. The combined ether extracts were evaporated and crystallized from ether, yield, 83 mg. (83%), m.p. 133-134°. The compound was found to be identical with 3-*O*-benzyl-4-pyridoxic acid lactone by mixed m.p. and its ir spectrum.

C. From 3-*O*-Benzylpyridoxol (IV).

3-*O*-Benzylpyridoxol (115 mg.) was added to the chromic oxide-pyridine-acetic acid complex solution (8) (8 ml.) and was shaken at room temperature for 22 hours. After working up as described under "B" (above), the ether extract on tlc (ethyl acetate) showed two spots (R_f = 0.5 (major spot) and 0.71 (minor spot)) and gave a Gibbs positive test after hydrolysis with 1*N* hydrochloric acid solution on the plate. The lower R_f (0.51) spot corresponded to that of 3-*O*-benzyl-4-pyridoxic acid lactone while the higher R_f (0.71) spot corresponded to that of 3-*O*-benzyl-5-pyridoxic acid lactone. By using preparative tlc (ethyl acetate) compounds corresponding to both spots were isolated and identified by mixed m.p., and comparison of their ir spectra. 3-*O*-Benzyl-4-pyridoxic acid lactone, yield, 72 mg. (63%), m.p. 134-135° (after crystallization from ether). 3-*O*-Benzyl-5-pyridoxic acid lactone, yield 24 mg. (22%), m.p. 113-114° (after crystallization from ether). Both of these compounds could also be separated by fractional crystallization from ether, since the 4-isomer is appreciably less soluble than the 5-isomer.

3-*O*-Benzyl-4-pyridoxic Acid Hydrazide.

Hydrazine (1 ml.) was added to a solution of 3-*O*-benzyl-4-pyridoxic acid lactone (100 mg.) in 5 ml. of alcohol and the reaction mixture was heated on a steam bath for 2 hours. After evaporation to dryness *in vacuo* the residue was crystallized from water or from alcohol-ether mixture, m.p. 138-139°, yield, 98 mg (88%); ir (Nujol): 3378 (NH), 1661 (CONH) cm^{-1}.

Anal. Calcd. for $C_{15}H_{17}N_3O_3$: C, 62.70; H, 5.96; N, 14.63. Found: C, 62.61; H, 6.10; N, 14.35.

3-*O*-Benzyl-4-pyridoxamide.

3-*O*-Benzyl-4-pyridoxic acid lactone (100 mg.) and liquid ammonia (10 ml.) were kept in a sealed tube at room temperature for 24 hours. After evaporation of ammonia, the residue weighed 97 mg. (90%) and was crystallized from alcohol-ether mixture, m.p. 195-196°; ir (Nujol): 3378 (OH), 3155 (NH$_2$), 1695, 1634 (C=O) cm^{-1}; nmr (DMSO): δ 4.57 (s, 5-CH$_2$), 4.97 (s, 3-*O*-CH$_2$), 7.45 (s, C$_6$*H*$_5$), 4.70, 4.82 (2xs, 4-CON*H*$_2$), 8.37 (s, C$_6$-*H*), 2-CH$_3$ peak is obscured in DMSO peak.

Anal. Calcd. for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29. Found: C, 65.87; H, 6.11; N, 10.58.

2-Methyl-3-benzyloxypyridine-4,5-dicarboxylic Acid Cyclic Imide (IX).

3-*O*-Benzyl-4-pyridoxamide (50 mg.) was dissolved in chromic oxide-pyridine-acetic acid complex solution (8) (5 ml.) and was shaken for ½ hour. After cooling in ice and dilution with water (25 ml.) it was extracted several times with ether. The combined ether extract was evaporated to dryness, water was added to the residue, and solid material separated out, which was washed with ice cold water, yield, 38 mg. (77%), m.p. 223-224° (after crystallization from methanol); ir (Nujol): 1718 (C=O) cm^{-1}; nmr (DMSO at 60°): δ 5.55 (s, 3-O-C*H*$_2$-), 7.48 (C$_6$*H*$_5$), 8.63 (C$_6$-*H*), 2-CH$_3$ is obscured in DMSO peak.

Anal. Calcd. for $C_{15}H_{12}N_2O_3$: C, 67.15; H, 4.51; N, 10.44. Found: C, 67.45; H, 4.57; N, 10.42.

2-Methyl-3-hydroxypyridine-4,5-dicarboxylic Acid (XIII).

2-Methyl-3-benzyloxypyridine-4,5-dicarboxylic acid imide (70 mg.) was dissolved in hydrochloric acid solution (1*N*, 20 ml.) and heated on a steam bath for 4 hours, and the solution was evaporated to dryness. Water was added and evaporated a few times to remove traces of acid. The solid residue was crystallized from boiling water after treatment with charcoal, yield, 36 mg. (72%), m.p. 260-261° dec.

Anal. Calcd. for $C_8H_7NO_5$: C, 48.74; H, 3.58; N, 7.11. Found: C, 48.52; H, 3.70; N, 7.96.

The compound was found to be in all respects identical with an authentic sample of the dicarboxylic acid kindly supplied by Dr. S. A. Harris of E. Merck and Co., Rahway, N. J.

2-Methyl-3-benzyloxypyridine-4,5-dicarboxylic Acid.

2-Methyl-3-benzyloxypyridine-4,5-dicarboxylic acid cyclic imide (IX, 70 mg.) was dissolved in alcoholic potassium hydroxide solution (5%, 15 ml.) and was heated on a steam bath for 2 hours. The alcoholic solution was evaporated completely *in vacuo*. The residue was taken up in water (15 ml.), cooled, acidified with acetic acid and evaporated. Water was added to the residue and evaporated again. This was repeated a few times to remove traces of acid, yield, 61 mg. (82%), m.p. 203-204° dec., after crystallization from alcohol; ir (Nujol): 3236 (OH), 1748, 1610 (C=O) cm^{-1}; nmr (1*N*, sodium deuterioxide): δ 2.40 (s, 2-C*H*$_3$), 4.98 (s, 3-O-C*H*$_2$), 7.43 (s, C$_6$H$_5$), 8.52 (s, C$_6$-*H*).

Anal. Calcd. for $C_{13}H_{13}NO_5 \cdot 0.5\ H_2O$: C, 60.81; H, 4.72; N, 4.72. Found: C, 60.42; H, 7.80; N, 4.72.

3-*O*-Benzyl-4-*N*,*N*-dimethylpyridoxamide (X).

3-*O*-Benzyl-4-pyridoxic acid lactone (VI, 150 mg.) and anhydrous liquid dimethylamine (20 ml.) were kept in a sealed tube at room temperature for 24 hours. After evaporation of dimethylamine, the residue was crystallized from ethylacetate-ether mixture, m.p. 141-142°, yield, 170 mg. (89%); ir (Nujol): 3401 (OH), 1629 (C=O) cm^{-1}; nmr (deuteriochloroform): δ 2.52 (s, 2-C*H*$_3$), 2.80, 3.08 (2xs, 4-CON (C*H*$_3$)$_2$), 3.85 (b, 5-O*H*), 4.57 (5C*H*$_2$), 4.90 (s, 3-O-C*H*$_2$-), 7.38 (s, C$_6$*H*$_5$), 8.37 (s, C$_6$-*H*).

Anal. Calcd. for $C_{17}H_{20}N_2O_3$: C, 67.98; H, 6.71; N, 9.33. Found: C, 67.81; H, 6.73; N, 9.12.

2-Methyl-3-benzyloxy-5-formylpyridine-4-*N*,*N*-dimethylamide (XI).

Method A. Oxidation with Chromic Oxide-Pyridine-Acetic Acid Complex Solution.

3-*O*-Benzyl-4-*N*,*N*-dimethylpyridoxamide (X, 50 mg.) was dissolved in glacial acetic acid (1 ml.), cooled in ice, chromic acid-pyridine-acetic acid complex solution (8) (1.2 ml.) was added and the reaction mixture was shaken vigorously for 3 minutes. After dilution with water (10 ml.) it was extracted several times with ether. The combined ether extract was washed with cold water, dried over anhydrous magnesium sulfate, and evaporated. The residue was extracted several times with petroleum ether (b.p. 37-54°) and the combined petroleum ether extract was evaporated *in vacuo* to a small volume, when a white crystalline material separated out. It was cooled, filtered and dried, yield, 36 mg. (72%), m.p. 86-87°; ir (Nujol): 1715 (C=O, aldehyde), 1650 (C=O, amide) cm^{-1}; nmr (deuteriochloroform): δ 2.60 (s, 2-CH_3), 2.77, 3.13 (2xs, 4-CON(CH_3)$_2$), 5.03 (3-O-CH_2), 7.42 (s, C_6H_5), 8.80 (C_6-*H*), 10.07 (s, 5-C*H*O).

Anal. Calcd. for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.33; H, 6.27; N, 9.68.

Method B. Oxidation with Manganese Dioxide.

3-*O*-Benzyl-4-*N*,*N*-dimethylpyridoxamide (X, 150 mg.) was dissolved in dry chloroform (20 ml.) and cooled in ice-water. Manganese dioxide (1.0 g., finely powdered) was added and the mixture stirred in the cold for 24 hours. After filtration (Celite) and washing with hot chloroform, the combined chloroform extract was evaporated to dryness *in vacuo*, and the residue crystallized from petroleum ether (b.p. 37-54°). Yield, 120 mg. (80%), m.p. 87°.

2-Methyl-3-hydroxy-5-formylpyridine-4-carboxylic Acid (I).

2-Methyl-3-benzyloxy-5-formylpyridine-4-*N*,*N*-dimethylamide (XI, 50 mg.) was dissolved in hydrochloric acid solution (1*N*, 50 ml.) and heated on a steam bath in an atmosphere of nitrogen for 3 hours, and the solution turned light yellow. It was evaporated completely *in vacuo*, water was added to the residue, and the process repeated a few times to remove traces of acid. The yellow precipitate that had separated out was cooled, filtered, washed with ice-cold water and dried, yield, 26 mg. (86%). It was further purified by dissolving in boiling water and treatment with a small amount of charcoal. On removal of most of the water *in vacuo* at room temperature, light yellow colored crystals separated out. The compound does not melt or decompose up to 320° but darkens at 245°; ir (Nujol): 2500 (OH), 1718 (C=O, carboxyl), 1575 sh (C=O, aldehyde) cm^{-1}.

Anal. Calcd. for $C_8H_7NO_4$: C, 53.04; H, 3.90; N, 7.73. Found: C, 52.77; H, 4.11; N, 7.87.

The compound was found to be identical with that of a sample of the natural metabolite, 2-methyl-3-hydroxy-5-formylpyridine-4-carboxylic acid, kindly supplied by Dr. E. E. Snell, by comparing its ir and uv spectra.

Semicarbazone Derivative of 2-Methyl-3-hydroxy-5-formylpyridine-4-carboxylic Acid.

Compound I (20 mg.) was dissolved in sodium bicarbonate solution (10%, 3 ml.), and a water solution of semicarbazide hydrochloride (15 mg. in 2 ml.) was added. The solution was kept at room temperature for 1 hour and then acidified with hydrochloric acid, when the semicarbazone precipitated. The latter was filtered, washed with cold water and dried, yield, 21 mg. (80%). It was further purified by dissolving in sodium hydroxide solution and reprecipitating with hydrochloric acid; it does not melt or decompose up to 320°.

Anal. Calcd. for $C_9H_{10}N_4O_4$: C, 45.38; H, 4.23; N, 23.52. Found: C, 45.09; H, 4.28; N, 23.75.

Oxime Derivative of 2-Methyl-3-hydroxy-5-formylpyridine-4-carboxylic Acid.

Compound I (30 mg.) was dissolved in 4 ml. of sodium carbonate solution (5%) and hydroxylamine hydrochloride (30 mg.) was added. The solution was kept at room temperature for 2 hours and acidified with hydrochloric acid. The precipitated oxime that separated out was filtered, washed with cold water, and dried; yield, 29 mg. (87%). It was further purified by dissolving in sodium carbonate solution (5%) and then reprecipitating with hydrochloric acid solution after treatment with charcoal, m.p. 211-212° dec.

Anal. Calcd. for $C_8H_8N_2O_4$: C, 48.98; H, 4.11; N, 14.28. Found: C, 49.25; H, 4.35; N, 14.31.

Diacetyl Derivative of 2-Methyl-3-hydroxy-5-formylpyridine-4-carboxylic Acid.

A mixture of the metabolite (I, 30 mg.), anhydrous sodium acetate (30 mg.) and acetic anhydride (2 ml.) was heated on a steam bath overnight (*ca.* 18 hours). The solution was evaporated completely *in vacuo*, the residue was cooled in ice, and crushed ice and ice-cold water were added, when the acetyl derivative precipitated out. It was filtered and washed with cold water, yield, 36 mg. (81%), m.p. 139-140° (from alcohol); ir (Nujol): 1808-1786 (C=O), 1245-1244 (C-O) cm^{-1}; nmr (DMSO-d_6): δ 2.22, 2.47 (2xs, acetyl), 2.55 (s, 2-CH_3), 7.58 (s, 4C*H* lactol), 8.90 (C_6-H).

Anal. Calcd. for $C_{12}H_{11}NO_6$: C, 54.34; H, 4.18; N, 5.28. Found: C, 54.37; H, 4.59; N, 5.34.

2-Methyl-3-benzyloxy-4-*N*,*N*-dimethylamidopyridine-5-carboxylic Acid (XII).

2-Methyl-3-benzyloxy-5-formylpyridine-4-*N*,*N*-dimethylamide (XI, 20 mg.) was dissolved in water (10 ml.) to which 2 drops of sodium hydroxide solution (0.1*N*) were added. Potassium permanganate (12 mg.) in water (2 ml.) was added drop by drop with stirring until the permanganate color was discharged. The reaction mixture was stirred at room temperature for an hour, and filtered after the addition of celite. The filtrate was acidified with acetic acid and evaporated in vacuo to a small volume, and then the acid precipitated out on cooling, yield, 14 mg. (66%), m.p. 180-181° (shrinks at 168-170°). When the oxidation was carried out in acetone, the yield was 71%.

Anal. Calcd. for $C_{17}H_{18}N_2O_4$: C, 64.95; H, 5.77; N, 8.91. Found: C, 64.26; H, 6.01; N, 8.46.

2-Methyl-3-hydroxypyridine-4,5-dicarboxylic Acid (XIII) from XII.

~~XII.~~

2-Methyl-3-benzyloxy-4-*N*,*N*-dimethylamido-5-carboxylic acid (XII, 14 mg.) was dissolved in 10 ml. of hydrochloric acid solution (1*N*) and heated on a steam bath for 3 hours. The acid solution was then evaporated to dryness *in vacuo*. Water was added to the residue and evaporated a few times to remove the traces of acid. The solid residue was crystallized from boiling water, m.p. 259-261° dec., yield, 6 mg. (75%).

The compound was found to be identical with that of a sample of dicarboxylic acid, prepared by acid hydrolysis of 2-methyl-3-benzyloxypyridine-4,5-dicarboxylic acid cyclic imide (IX as described above), by mixed m.p. and comparison on tlc (1:1 dioxane and water: R_f = 0.8).

3-*O*-Benzyl-5-pyridoxic Acid Lactone (XIV).

A solution of 5-pyridoxic acid lactone (2.0 g.) in absolute alcohol (50 ml.) was reacted with dimethylphenylbenzylammonium hydroxide (obtained by treatment of dimethylphenylbenzylammonium chloride (3.5 g.) in 25 ml. of alcohol with sodium metal (0.33 g.) in 15 ml. of absolute alcohol) under similar conditions used for the synthesis of 3-*O*-benzyl-4-pyridoxic acid lactone (VI, see above), yield, 2.1 g. (68%), m.p. 113-114° (after crystallization from ether); ir (Nujol): 1770 (C=O) cm^{-1}; nmr (deuteriochloroform): δ 2.65 (s, 2-CH_3), 5.13 (s, 5-CH_2), 5.17 (s, 3-O-CH_2), 7.40 (s, C_6H_5), 8.78 (s, C_6-*H*).

Anal. Calcd. for $C_{15}H_{13}NO_3$: C, 70.58; H, 5.13; N, 5.49. Found: C, 70.36; H, 5.20; N, 5.65.

3-*O*-Benzyl-5-*N*,*N*-dimethylpyridoxamide (XV).

3-*O*-Benzyl-5-pyridoxic acid lactone (XIV, 0.6 g.) and anhydrous liquid dimethylamine (*ca.* 20 ml.) were kept in a sealed tube at room temperature for 24 hours. After evaporation of dimethylamine, the residue was crystallized from ethyl acetate, yield, 0.65 g. (92%), m.p. 128-129°; ir (Nujol): 3205 (OH), 1658 (C=O) cm^{-1}; nmr (deuteriochloroform): δ 2.53 (s, 2-CH_3), 3.00, 3.15 (2xs, 5-CON (CH_3)$_2$), 3.82 (b, 4-O*H*), 4.62 (s, 4-CH_2), 5.03 (s, 3-O-CH_2), 7.42 (s, C_6H_5), 8.23 (s, C_6-*H*).

Anal. Calcd. for $C_{17}H_{20}N_2O_3$: C, 67.98; H, 6.71; N, 9.33. Found: C, 68.14; H, 6.91; N, 9.05.

2-Methyl-3-benzyloxy-4-formylpyridine-5-*N*,*N*-dimethylamide (XVI).

A. Oxidation by Chromic Oxide-Pyridine-Acetic Acid Complex Solution.

3-*O*-Benzyl-5-*N*,*N*-dimethylpyridoxamide (XV, 50 mg.) in 1 ml. of glacial acetic acid was oxidized by chromic oxide-pyridine-acetic acid complex solution (8) (1 ml.) under similar conditions used for the synthesis of 2-methyl-3-benzyloxy-5-formylpyridine-4-*N*,*N*-dimethylamide (XI). After working up, the white crystalline compound was obtained from a concentrated petroleum ether (b.p. 37-54°) extract, filtered and dried. It weighed 32 mg. (64%), m.p. 90-92°; ir (Nujol): 1701 (C=O aldehyde), 1658 (C=O amide) cm^{-1}; nmr (deuteriochloroform): δ 2.63 (2-CH_3), 2.73, 3.13 (2xs, 5-CON (CH_3)$_2$), 5.07 (3-O-CH_2), 7.38 (s, C_6H_5), 8.32 (s, C_6-*H*), 10.25 (4-C*H*O).

Anal. Calcd. for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.64; H, 6.27; N, 9.57.

B. Oxidation by Manganese Dioxide.

3-*O*-benzyl-5-*N*,*N*-dimethylpyridoxamide (XV, 80 mg.) was dissolved in 15 ml. of dry chloroform (over drierite) and cooled in ice. Manganese dioxide "B" (550 mg., finely powdered) was added all at once and stirred in the cold for 24 hours. Filtration after the addition of celite and washing several times with hot chloroform, evaporation and crystallization from petroleum ether (b.p. 37-54°) yielded 60 mg. (75%) of XVI, m.p. 93-94°.

The compound was identical with that obtained by method A.

The product obtained by manganese oxide oxidation is of superior quality and is free from contaminants. The product obtained by treatment with chromic-oxide-pyridine-acetic acid complex solution is sometimes contaminated with 3-*O*-benzyl-5-pyridoxic acid lactone, indicating that during oxidation of 4-CH_2OH to 4-CHO, the 5-amido group is hydrolyzed to some extent.

2-Methyl-3-hydroxy-4-formylpyridine-5-carboxylic Acid (XVII).

2-Methyl-3-benzyloxy-4-formylpyridine-5-*N*,*N*-dimethylamide and heated on a steam bath in an atmosphere of nitrogen for 4 hours. The yellow solution was evaporated to dryness *in vacuo*, water was added, and the evaporation was repeated a few times to remove traces of acid. Unlike the metabolite (I), the compound did not crystallize out from solution, appeared to be photo-sensitive and decomposed slowly on further attempts at purification. The compound was characterized by the following derivatives.

Semicarbazone of XVII.

Compound XVII (obtained by hydrolysis of XVI with hydrochloric acid (1*N*, 50 ml.), 100 gm.) was dissolved in sodium bicarbonate solution (10%, 12 ml.) and cooled in ice. Semicarbazide hydrochloride (42 mg.) was added. Keeping at room temperature for 2 hours and then acidifying with hydrochloric acid caused the semicarbazone to separate out. After filtration it was further purified by dissolving in sodium hydroxide solution (1*N*) and reprecipitation with hydrochloric acid, m.p. 220-221° dec.; ir (Nujol): 3509, 3413, 3279 (OH, NH), 1701, 1672, 1618, 1575 (C=O) cm^{-1}.

Anal. Calcd. for $C_9H_{10}N_4O_4$: C, 45.39; H, 4.23; N, 23.53. Found: C, 45.06; H, 4.39; N, 23.36.

Diacetyl Derivative of XVIIB.

Compound XVII (obtained by hydrolysis of XVI with hydrochloric acid (1*N*, 50 ml.), 100 mg.) was dissolved in acetic anhydride (5 ml.) and anhydrous sodium acetate (100 mg.) and heated on a steam bath overnight (*ca.* 18 hours). The reaction mixture was evaporated completely *in vacuo*, and the residue was extracted several times with ether. After removal of ether the residue was crystallized from ether-petroleum ether extract, yield, 46 mg. (52%), m.p. 109-110°; ir (Nujol): 1783, 1634 (C=O) cm^{-1}.

Anal. Calcd. for $C_{12}H_{11}NO_6$: C, 54.34; H, 4.18; N, 5.28. Found: C, 54.13; H, 4.31; N, 5.38.

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latter compound to carboxylic acid or to benzylate its 3-phenolic hydroxyl group with "leucotrope" was not fruitful and gave a mixture of products. Similarly an effort to oxidize the 5-hydroxymethyl group to 5-formyl in 3-*O*-4-pyridoxic acid hydrazide by treatment with dicyclohexylcarbodiimide (DCC), DMSO and phosphoric acid was not successful.

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